

**REMARKS/ARGUMENTS**

Reconsideration of the above-identified application is respectfully requested in view of the amendments to the claims and the following remarks.

Claims 4, 7 and 10 have been cancelled.

Claims 1-3, 5, 8-9, 11-13, 15, 17-20, 23, and 26-30 have been amended.

Claims 33-34 have been withdrawn.

Claims 1-3, 5-6, 8-9, and 11-32 remain in the case.

No revision of inventorship is necessary in the present application.

**Support for the Amendment**

Support for amended claims is found throughout the specification and in the original claims. Specific support is shown below.

Support for inclusion of the term "relative concentration defined by the concentration that is sufficient to exceed threshold of assay to known amounts of antifreeze protein" in amended Claim 1 is found in original Claim 1 and specification page 102, lines 21-31, through page 103 lines 7-15.

Support for inclusion of the term "by means of imaging" in amended Claim 1 is found in original Claim 13.

Support for inclusion of the term "quantitative and statistical analysis" in amended Claim 1 is found in original Claims 14 and 15.

Support for inclusion of the term "purified Tm 12.84" in amended Claim 5 is found in the specification at page 14, lines 23-38.

Support for inclusion of the term "in a concentration from about" in amended Claim 8 is found in the specification at page 17, lines 21-15, and in the original Claim 8.

Support for amended Claim 11 is found in original Claims 10 and 11.

Support for inclusion of the term "showing log(dilution)" in amended Claim 23 is found in the original Claim 19.

Support for inclusion of the term "predetermined amount" in amended Claim 26 and amended Claim 27 is found in the specification at page 17, lines 11 through page 18, line 13, and original Claims 26-27.

Support for inclusion of the term "in a range of approximately 2 - 4°C below a melting point of said test sample" in amended Claim 30 is found in original Claim 30, and in the specification at page 81, line 30 through page 82 line 36.

### Invention Summary

Thermal hysteresis proteins (THPs) also known as antifreeze proteins are known to lower the non-equilibrium freezing point of water without lowering the melting point (equilibrium freezing point). The present invention details recrystallization inhibition (RI) behavior of thermal hysteresis proteins. In particular, extremely dilute solutions of THPs have been shown to inhibit the recrystallization of fine-grained ice samples in a concentration-dependent manner. The high sensitivity of RI to the presence of THPs led Applicants to the present invention, as recited in the amended claims, which is a quantitative assay of THP activity using the recrystallization inhibition behavior. The extent of recrystallization in a fine-grained ice sample is quantified by estimating mean largest cross-sectional area for ice grains in the sample, thus providing the basis for a numerical assessment of RI. A number of different assay characteristics is addressed and described in the specification, including specificity of the RI assay with respect to THP, ice grain size homogeneity within RI ice samples, RI assay sensitivities, applications of the assay, and assay automation.

As defined in currently amended claim 1, the invention particularly defines a RI method for determining the presence, relative concentration, and activity of THPs. A test solution made of a proteinaceous composition in a solvent is flash frozen; the temperature of the frozen solution is raised to an appropriate annealing temperature that allows for a partial

melt, while limiting heterogeneity in ice grain sizes within the solution. The frozen solution is maintained at the annealing temperature for a length of time sufficient to allow for ice recrystallization. Changes in ice crystal grain size are monitored over time; and the presence of functional THPs (while reducing the effect of non-specific proteins) in the solution is determined by measuring ice crystal grain sizes relative to a control solution.

### Claim Objections

Claims 2, 10-13, 15, and 17-19 were objected to by the Examiner.

Claim 2 has been amended to supply a missing "is" from the claim.

Claim 5 has been amended for clarity.

Claims 10 and 11 were combined as suggested by the Examiner. Claim 10 has been cancelled. Claim 12 now depend from Claim 11.

Claim 13 has been amended to incorporate the Examiner's suggested claim language.

Claim 15 and Claim 17 have been amended to insert the full terms represented by the abbreviations as required by the Examiner.

Claim 17 has been amended to remove ambiguous recitation of "and/or."

Claim 18 was amended to remove the extra parenthesis noted by the Examiner.

Claim 29 has been amended for clarity.

The claims have been further amended to help clarify the subject matter of the invention.

Applicants now believe that the foregoing amendments to the claims overcome the objections.

**Claim Rejections 35 U.S.C. §112**

Claims 1-32 were rejected under 35 U.S.C. §112, second paragraph.

Claim 1 has been amended to remove the ambiguous term "and or" in the preamble. Claim 1 has been amended to clarify "relative concentration"; include "proteinaceous composition", "recrystallization inhibition (RI) factor". Specifically the last step is amended to include positive recitation of terms to correlate "presence, relative concentration and activity" in terms of quantitative and statistical determination. The rejection of Claim 1 is therefore believed to have been overcome.

Claim 2 has been amended to delete the recitation of "other isoosmotic inorganic or organic solutions".

Claim 6 was rejected for providing a Markush listing of proteinaceous compounds inconsistent with the preamble of claim 1. The amendment of claim 1 is believed to remove any inconsistency between the Markush group of claim 6 and the preamble of claim 1. Its rejection is, therefore, believed to have been overcome.

Claim 7 has been cancelled.

Claim 8 has been amended to delete the term "includes" and has been replaced by "comprises".

Claim 9 has been amended to replace "the" with "a" and now depends from Claim 1. Applicants believe that the lack of antecedent basis rejection has been now overcome.

Claim 26 has been amended to remove "known characterized parameters experimentally measured" and "predetermined concentration" is added along with other amendments for clarity. Claim 27 has been amended to replace "known" with "predetermined".

Claim 30 was rejected because the term "high annealing temperature" is a relative term. Claim 30 has been amended to positively recite an annealing temperature of approximately 2-4°C below the melting point of the test solution.

Applicants believe that with this amendment of these claims the 35 U.S.C. §112, second paragraph rejection of Claims 1-32 has been overcome.

**Claim Rejections 35 U.S.C §103**

Claim 1 was rejected under 35 U.S.C. §103(a) as being unpatentable over Olien (Ann. Rev. Plant Physiology, vol. 18, pages 337-408, 1967) in view of United States Patent No. 5,118,792 for ICE CRYSTAL GROWTH SUPPRESSION POLYPEPTIDES AND METHOD OF MAKING, issued June 2, 1992 to Warren et al. Applicants respectfully traverse this ground of the rejection.

The Examiner acknowledges that Olien does not explicitly teach a method for determining the presence of hysteresis protein. The Examiner maintains that "this method is a measure of recrystallization inhibition" because the Olien method for plant extracts involves an initial freezing, followed by thawing to about three fourths (partial thaw, so that some ice remains at the start of the refreezing bout to nucleate and avoid supercooling), and then the sample is refrozen slowly. See Olien at 396. The subsequent refreezing of the remaining liquid water is then monitored and kinetics of heat removal evaluated over a series of re-freezing tests with different freezing rates during the refreezing process.

However, Applicants note the Olien method is neither an assessment of recrystallization inhibition, nor of the process of recrystallization itself. Rather, as Olien states, it is an assessment of a refreezing process at various temperatures (not a thawing process as would be the case for recrystallization).

Unlike Olien, the present invention involves a RI method for determining the presence, relative concentration and activity of thermal hysteresis proteins by raising the temperature and allowing the frozen test solution to obtain a partial melt and not an equilibrium freezing process. The basic procedure that Olien presents (i.e., monitoring ice crystal growth in a film during a "refreezing" process, involving first a partial thaw, and then a bout of refreezing) is the basic concept behind two, current day methods for assessing thermal hysteresis: the nanoliter osmometer, requiring microscopic observations (Chakrabartty and Hew, 1989; J. Biological Chemistry, Volume 264, pp. 11313-11316), and the rather sophisticated and costly thermodynamic assessments involving differential scanning calorimetry (DSC) (Hansen and Baust, Biochimica et Biophysica Acta. Vol. 957, pages 217-221, 1988). Again, as with Olien, these current methods are evaluating an equilibrium freezing process, and not the phenomena of recrystallization, or its inhibition.

Furthermore, Olien, does not teach the quantitative Relative Inhibition (RI) method of the present invention for assay which is specific for antifreeze proteins that exhibit RI effects. (See specification Claim 1.) In fact Olien is silent to the effect of RI contribution of non-antifreeze specific proteins. Applicants have demonstrated, using RI factors, the sensitivity of the method for non-antifreeze specific proteins. (See Claim 1 and Claim 22 specification).

Therefore, Olien teaches away from the present invention.

The Examiner states that Warren discloses screening the antifreeze polypeptides by monitoring inhibition of ice crystal growth via refreezing on a cooled metal block, the splat assay. See Warren at column 4, lines 48-62 and example 3.

Warren uses the splat assay to screen for antifreeze proteins (AFPs) via assessing the extent of recrystallization occurring in a frozen sample. Any RI effects, presumably attributed to the presence of AFPs, is the basic premise behind the need for the present invention.

Applicants note that Warren does not account for AFP specificity. That is, there are numerous circumstances under which the splat assay gives a false positive indication for the presence of AFPs, when in fact no AFPs are present. Moreover, Warren's results are obtained subjectivity by visual monitoring of ice crystal size changes and lack the statistical and quantitative approach of the present invention.

Moreover, Warren is silent on sensitivity of the present invention. Applicants have detected THP activity in the 0.1ug/ml range (see specification at page 101, lines 16-34) in contrast to 0.1 mg/ml range of Warren (see Warren at Col. 18, lines 31-34), thus the present invention is three orders of magnitude more sensitive than the Warren method.

Also, Warren merely mentions the splat assay method. Applicants note that reasonable expectation of success is required for motivation to combine and some degree of predictability is required and not the mere suggestion as the Examiner has asserted. (See MPEP §2143.02.)

Therefore, Applicants believe that there would be no motivation to combine the teaching of Olien with Warren to obtain the method of the present invention.

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Even if such motivation were present, Warren does not account for superior results of the present invention. For example the quantitative approach of applicants using mean large grain size obtained by quantitative and statistical analysis of imaging data have found that results were superior in terms of having lesser variability than the results obtained by using light scattering techniques. Coefficient of variation by regression analysis is higher for mean large grain size analysis (0.941) compared with light scattering (0.853). See specification at page 89, lines 26-30. Certainly, the visual and subjective analysis of Warren, would not be expected to yield such a superior result.

In Applicants' opinion, the Office has not made a prima facie case of obviousness; as a result, the rejections of claim 1 have been successfully traversed.

### Conclusion

In view of the above arguments and amendments to the claims, Applicants believe they have overcome both the indefiniteness and obviousness rejections. Claims objections also have been overcome. Applicants request reconsideration of this application and allowance of pending claims 1-3, 5-6, 8-9, and 11-32.

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